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THE PRECIPITATION OF COLLOIDAL GOLD IN THE CEREBROSPINAL FLUID OF HORSES WITH DOURINE

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Dourine is a trypanosomiasis which, under natural conditions, affects only the horse and the ass.¹ The cause, *Trypanosoma equipedum*, is transmitted solely by coition, and two distinct stages are noted, the local lesions on the genital organs followed by an affection of the nervous system, indicated usually by a paralysis of the posterior extremities, although occasionally the forelegs and the face are affected.

Dourine was first recognized in the United States in 1886, and in spite of vigorous control measures, it continued to make its appearance from time to time in different parts of the country. Until 1912, the diagnosis of the disease rested solely on physical examination which, due to the nature of the malady, was unsatisfactory as the disease would be detected only in animals showing clinical symptoms at the time of examination. At this time Mohler, Eichhorn and Buck² perfected the application of the complement-fixation reaction to the diagnosis of dourine, and by its means the extent of the disease was definitely determined.

An active campaign was then begun, by the Bureau of Animal Industry and the various states in which dourine existed, for the control and eradication of the disease, the complement-fixation test being adopted as the official diagnostic agent.

Samples of blood serum from all breeding animals in territory in which dourine exists, or is suspected, are forwarded to the pathologic laboratory of the Bureau of Animal Industry where they are subjected to the complement-fixation test. The animals are held in virtual quarantine until the results of the test are received, and only those animals

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¹ Mohler and Schoening: Dourine of Horse, Farmers' Bull. 1146, U. S. Dept. of Agric.

² Proceedings Am. Vet. Assn., 1913.

whose serums give a negative reaction are released for breeding. Animals whose serums give a positive reaction are destroyed and their owners reimbursed.

The greater part of the work is done in the spring and early summer and large numbers of samples are tested daily—as high as 1,800 samples have been tested in one day. Special apparatus³ designed to facilitate this work are factors in making possible the testing of large numbers of specimens with a minimum of labor and no confusion.

In view of the fact that the colloidal gold test devised by Lange,⁴ has been used with a large degree of success in classifying affections of the central nervous system, and the further fact that dourine presents, in certain stages of the disease, symptoms of paralysis as a result of degeneration of the peripheral nerves and the intervertebral ganglions, it was thought that this test might give results of scientific interest, if not practical value, when applied to the spinal fluid of infected horses.

Accordingly, we concluded to select certain animals, the blood serum of which reacted to the complement-fixation test for dourine, and obtain from them specimens of spinal fluid for examination. The specimens were collected immediately after killing the animals.

The 33 specimens of spinal fluid which were suitable for examination were subjected to the complement-fixation test for dourine, the fluids being tested in 3 amounts, 0.1, 0.25, and 0.5 cc, with an antigen⁵ composed of pure trypanosomes preserved in glycerol. Globulin tests were made according to the Ross-Jones method. The remaining fluid was then centrifuged, the lower stratum turned out on a slide, fixed and stained according to Wright's method, and examined for trypanosomes, but in no case were any found. Owing to the disintegration of cells, no reliability could be placed in cell counts. The limited amount of spinal fluid did not permit an examination for protein content. Table 1 gives the results.

In noting the results of the colloidal gold test, five reactions were recognized:

5. Supernatant fluid colorless; precipitation of gold complete
4. Pale blue
3. Blue
2. Purple or lilac
1. Red blue
0. No change

³ Buck, J. M.: *Jour. Infect. Dis.*, 1916, 19, p. 267. Reynolds, F. H.: *Jour. Agric. Research*, 1918.

⁴ Berl. klin. Wchnschr., 1912, 49, p. 897; *Ztschr. f. Chemotherapie*, 1912, 1, p. 44.

⁵ Reynolds, F. H., and Schoening, H. W.: *Jour. Agric. Research*, 1918.

TABLE 1
COLLOIDAL GOLD TEST OF CEREBROSPINAL FLUID OF HORSES WITH DOURINE

Groups	Nos.	Globulin Test	Complement Fixation		Clinical Symptoms and Postmortem Findings	Colloidal Gold Test of Cerebrospinal Fluid
			Serum	Spinal Fluid		
Horses showing clinical evidence of Dourine—7	1	Neg.	0.07 c c pos.	0.1 c c \pm 0.25 c c 4+	Scars on vulva	0012321100
	2	Pos.	0.03 c c pos.	Neg.	Scars on vulva, slight posterior paralysis	0050000000
	3	Trace	0.05 c c pos.	Neg.	Scars on penis and prepuce	0455543210
	4	Neg.	0.01 c c pos.	Neg.	Facial paralysis	1112343200
	5	Trace	0.08 c c pos.	Neg.	Depigmented spots on scrotum and ulcers on penis	0012200420
	6	Neg.	0.07 c c pos.	0.25 c c 1+ 0.5 c c anti-complementary	Numerous skin eruptions and posterior paralysis	0014520021
	7	Neg.	0.03 c c pos.	Neg.	Ulcers on ext. genitalia, incoordination of hind legs	0012344222
Horses giving fixation but no clinical symptoms of Dourine—6	1	Trace	0.02 c c pos.	0.25 c c 4+	None	0000000000
	2	Pos.	0.005 c c pos.	0.1 c c 1+ 0.25 c c 4+	None	0001100000
	3	Trace	0.02 c c pos.	0.1 c c neg. 0.25 c c 4+	None	0001110000
	4	Pos.	0.08 c c pos.	0.1 c c 2+ 0.25 c c anti-complementary	None	0001110000
	5	Neg.	0.01 c c pos.	0.1 c c neg. 0.25 c c 4+	None	0011311100
	6	Trace	0.07 c c pos.	0.1 c c neg. 0.25 c c 2+	None	0001222511
Horses without clinical symptoms, the spinal fluid not giving the fixation test—18	1	Neg.	0.005 c c pos.	Neg.	None	0000110000
	2	Trace	0.02 c c pos.	Neg.	None	0000200000
	3	Neg.	0.005 c c pos.	Neg.	None	0001210000
	4	Neg.	0.1 c c pos.	Neg.	None	1112210000
	5	Trace	0.01 c c pos.	Neg.	None	0012321100
	6	Neg.	0.08 c c pos.	Neg.	None	0155432100
	7	Neg.	0.06 c c pos.	Neg.	None	0355320000
	8	Pos.	0.01 c c pos.	Neg.	None	0000011100
	9	Trace	0.07 c c pos.	Neg.	None	0000011110
	10	Neg.	0.005 c c pos.	Neg.	None	0012334111
	11	Neg.	0.2 c c pos.	Neg.	None	0001012000
	12	Neg.	0.005 c c pos.	Anticomplementary	None	0001203100
	13	Neg.	0.02 c c pos.	Neg.	None	001332140
	14	Neg.	0.05 c c pos.	Neg.	None	0000120550
	15	Neg.	0.005 c c pos.	Neg.	None	0124111000
	16	Neg.	0.02 c c pos.	Neg.	None	0014413110
	17	Neg.	0.2 c c pos.	Neg.	None	0124411100
	18	Neg.	0.005 c c pos.	Neg.	None	4111222111
Colloidal gold test neg.—2	1	Neg.	0.03 c c pos.	Neg.	None	0000000000
	2	Trace	0.2 c c pos.	Neg.	None	0000000000
Normal control	..	Neg.	Neg.	Neg.	None	0000000000

Of the 7 horses showing clinical evidence of dourine, 1 was markedly positive for globulin, 2 showed a trace and 4 were negative. The 3 showing the presence of globulin were negative to the fixation test. Of the 4 which were negative for globulin, 1 showed a 4+ fixation in 0.25 cc of fluid, a 1+ in 0.25 cc, while the remaining 2 were negative to the test.

As it was impracticable to procure the cords from these horses for microscopic examinations, it is not possible to place conclusive interpretations on the reactions to the colloidal gold test but, in passing, attention is called to the similarity of the reactions in Nos. 1, 2, 3, and 4 to those which might be suggestive of syphilis in man and of No. 5 to a curve as indicative of cerebrospinal syphilis (Kaplan⁶), while that of No. 7 is somewhat similar to the reaction obtained in cases of meningitis in man.

In the 6 tests, of spinal fluid from horses presenting no visible lesions but giving various degrees of fixation, 2 were markedly positive for globulin, 3 slightly so and 1 negative. Of those positive for globulin, 2 gave 4+ fixations with 0.25 cc of fluid, 2 gave 2+ fixations with the same amount while 1 gave a 1+ with 0.1 cc. No. 5, which was negative to the globulin test, gave a 4+ fixation with 0.25 cc of fluid. No. 1, which gave a 4+ fixation and showed a trace of globulin, failed to react in any degree to the colloidal gold test. Were the peaks higher in Nos. 2, 3, 4, and 5, the reactions would be similar to those obtained in cases of spinal syphilis. No. 6 gave a curve not unlike that of meningitis, the reduction peak being almost to the extreme right.

In the case of the horses whose spinal fluids failed to react to the complement-fixation test, the donors presenting no visible lesions of dourine, 2 were positive for globulin while 3 showed a trace; the remaining 13 were negative. In 17 the spinal fluid was negative to the complement-fixation test and in 1 it was anticomplementary. It is interesting to note that the 5 which were positive for globulin, are rather subdued in their reactions to the colloidal gold test. Nos. 2 and 3 have their peaks at 2 on the scale, and in the center; No. 5 has its peak at 3 and in the center; while Nos. 8 and 9 rise to 1, are at the right of the center, and produce nearly the same degree reaction.

Of the 2 fluids that gave no reaction to the gold, one showed a trace of globulin. Whether there were alteration of the cord and

⁶ Serology of Mental and Nervous Diseases, 1914.

meninges is not known. According to Wood, Vogel, and Famulener,⁷ a normal spinal fluid always gives a straight line, but a straight line does not necessarily indicate that the cord is normal, for myelitis in man has given 10 zeros.

SUMMARY

The colloidal gold test, when applied to the spinal fluids of horses suffering with dourine, in many instances gives reactions of varying intensity. A number of the reactions are similar to those in cases of cerebrospinal syphilis in which the peak of the curve is found at about the center of the scale. In some instances, the reactions recorded attained a height of 5. In other cases the reactions showed a tendency to rise and stopped abruptly at 1, 2 and 3, then dropped to zero. This suggests the possibility that a more energetic curve might have been attained had the disease progressed to a greater degree before the destruction of the animal. Some of the curves are similar to those in meningitis, while no reactions of the paretic type were obtained.

While reactions were obtained with the colloidal gold test, just what they may indicate cannot now be stated in the absence of careful study of the spinal cords. However, in the presence of a positive serum test and in some cases of clinical evidence, it would appear that the reactions would be of some significance.

In many cases there appeared to be no agreement between the serum fixation, fluid fixation and the globulin and colloidal gold tests; but when consideration is given the active campaign against this malady and the rapid destruction of animals reacting to the serum fixation tests, and the remote chance of the disease progressing to any great length, it is not surprising that so few reactions to the fixation tests were obtained with spinal fluid. The apparent absence of globulin in many cases is not so disconcerting, as the test employed may not have recorded its presence sufficiently to make it discernible macroscopically; further, other proteins, not detected by the method at hand, may have influenced the colloidal gold reactions.

Several specimens of spinal fluid gave the complement-fixation test for dourine.

⁷ Laboratory Technique, 1917.